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Immunoluminometric Assays for the Quantification of Apolipoproteins A-I, B, C-II, Apolipoprotein(a) and Lipoprotein(a)

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Summary: Immunoluminometric assays were developed for apolipoproteins A-I, B, C-II (as the apolipoprotein C-II : apolipoprotein B complex), apolipoprotein(a) and lipoprotein(a). The assays were evaluated clinically and methodologically. The results for apolipoprotein A-I, apolipoprotein B and lipoprotein(a) were compared with those obtained by turbidimetric assays. No comparison was possible for apolipoprotein C-II.

Sample predilution was necessary for apolipoprotein A-I, apolipoprotein B, apolipoprotein(a) and lipoprotein(a). Not all antibody combinations gave rise to assays with acceptable recovery rates. This was especially the case for lipoprotein(a).

These assays for apolipoprotein(a) and lipoprotein(a) had relatively low detection limit (< 5 mg/l) and measuring ranges up to 800 mg/l (using a 1: 10 sample dilution), and were suitable for routine use, especially for longer series.

Assay times were all less than 3 h, each assay using the streptavidin-biotin technique and being based on a coated-ball technology.

The median intra-assay coefficients of variation for all assays lay between 2.9 and 5.9% in the range of interest, expressed in terms of precision profiles. Inter-assay (im)precision lay between 6.2 and 12.2%, calculated in the accepted way.

Correlations between turbidimetric and immunoluminometric assays for apolipoprotein A-I and apolipoprotein B were statistically acceptable, although the correlation coefficients were mediocre (apolipoprotein A-I – r = 0.65, apolipoprotein B – r = 0.83) and the slope of the regression line differed from unity. This was most probably due to standardisation and differences in assay design (one-site competitive versus two-site immunometric assays).

The correlation between the turbidimetric and immunoluminometric assays for lipoprotein(a) (r = 0.87) and apolipoprotein(a) (r = 0.89) were good, although again, the slope of the regression line differed from unity, which was probably not due to free apolipoprotein(a) but to serum matrix effects, as the same standard was used in both assays.

The assays were suitable for routine use, especially when longer series were run. The costs were considerably lower than those for turbidimetric determinations. The high dilution factor for apolipoprotein A-I (up to 1 : 2000) and apolipoprotein B (up to 1 : 1000) could be seen as a disadvantage of the immunoluminometric assays.

Introduction

The introduction of apolipoprotein determination in addition to the "classical" lipid metabolism quantities, such as triacylglycerols and different cholesterol fractions, has improved the armamentarium available for the surveillance, prevention and combatting of atherosclerosis and coronary heart disease (1–10). Lipoprotein(a) has been shown to be an important independent marker for atherosclerosis; it is closely related to plasminogen (11–

15), thus providing a possible link between lipid metabolism and haemostaseology.

Most methods for determination of apolipoproteins are based on turbidimetry or nephelometry, as the relatively high concentrations in blood do not necessarily demand methods with a very low detection limit. The determination of lipoprotein(a) using turbidimetry or nephelometry has proved problematic with regard to sample type (serum or plasma) and reproducibility.

Immunometric technology allows the specific determination of defined apolipoproteins, as well as enabling the determination of particles containing different apolipoproteins. The main disadvantage of the method is the dilution step often needed prior to assay. This is compensated by the application of the method to long series, for example in screening procedures. This technology provides a reliable and specific quantification of analytes that are present in blood in low concentrations, in the present case apolipoprotein C-II complexed with apolipoprotein B.

All assays are based on commercially available reagents and can be set up in any laboratory with suitable measuring equipment. Since the methods use biotinylated antibodies and labelled streptavidin, they can also be adapted for radioisotopic, enzyme or time-resolved fluorescence markers.

Materials and Methods

Materials

Antibodies were purchased from the following sources:

Dako, Hamburg, Germany,
Immuno, Heidelberg, Germany,
Atlantic Antibodies (ATAB) (Serva), Heidelberg, Germany,
Boehringer-Mannheim, Mannheim, Germany,
Calbiochem-Novabiochem, Bad Soden, Germany.

The combination and source of the antibodies for each assay is given below.

Standard and control materials were obtained from Boehringer-Mannheim, Immuno, Behringwerke, Marburg a. d. L., Germany, Roche Diagnostics, Grenzach-Wyhlen, Germany and Dako.

Amidocaproylbiotin N-hydroxysuccinimide was purchased from Sigma, Deisenhofen, Germany. Streptavidin was obtained from Biomol, Hamburg, Germany. 9-[N-(4-aminobutyl)-N-ethyl] aminobenzo(f)phthalazine 1,4 (2H,3H) dione (ABEN) was synthesised in the Institute for Biochemical Endocrinology of the Medical University of Lübeck.

Polystyrene balls (6.4 mm diameter) were obtained from Spherotech Kugeln GmbH, Fulda, Germany.

Buffer substances were purchased from Merck, Darmstadt, Germany or from Sigma.

Methods

The biotinylation of antibodies and labelling of streptavidin with ABEN was carried out as previously described (16, 17).

Tab. 1 Assay scheme for the immunoluminometric determination of apolipoproteins.

10 µl sample (neat for apolipoprotein C-II, or diluted for apolipoprotein A-I, apolipoprotein B and (apo)lipoprotein(a))
200 µl assay buffer
1 antibody coated ball (anti apolipoprotein C-II, anti apolipoprotein A-I, anti apolipoprotein B or anti apolipoprotein(a))
Incubate on horizontal shaker (170 min^{-1}) for 60 min Wash with 2 × 5 ml demineralised water
200 µl biotinylated antibody (anti apolipoprotein A-I, anti apolipoprotein B or anti apolipoprotein(a))
Incubate and wash as above
200 µl streptavidin-ABEN conjugate
Incubate for 30 min and wash with 3 × 5 ml demineralised water.
*) Transfer ball to measuring cuvette, add 300 µl catalase, load luminometer and initiate reaction with 300 µl alkaline peroxide, integrating the light signal over 4 seconds.

*) Full details of the reagents for luminometry have been published in detail elsewhere – see l. c. (16, 17).

The assay scheme was the same for all analytes and is shown in table 1. Comparative methods were carried out on the Cobas MIRA (Roche Diagnostics) using turbidimetric methodology. The standards and antiserum used were from Immuno for lipoprotein(a), and from Roche for apolipoprotein A-I and B for turbidimetry. The standards for all five immunoluminometric assays were all from Immuno.

The antibody combinations for the immunoluminometric assays developed were as follows:

Apolipoprotein A-I: Solid phase and biotinylated, Dako (Code No. Q 496).

Apolipoprotein B: Solid phase and biotinylated, Dako (Code No. Q 497).

Apolipoprotein C-II: Solid phase, Calbiochem anti-apolipoprotein C-II, biotinylated anti-apolipoprotein B, DAKO (Code Q-497).

Lipoprotein(a): Solid phase, Dako anti-lipoprotein(a) (Code No. Q 023), biotinylated Dako anti-apolipoprotein-B (Code No. Q 497).

Apolipoprotein(a): Solid phase, Dako anti-lipoprotein(a) (Code No. Q 023), biotinlated, Immuno anti-lipoprotein(a). Both antibodies were raised against apolipoprotein(a).

Apolipoprotein A-I

The best results were obtained with the combination of the antibodies shown above. The sample was assayed at dilutions of 1 : 500, 1 : 1000 and 1 : 2000. The standard curve covered the concentration range 0.125–2 mg/l (effective range up to 4 g/l). The standard had been calibrated against the proposed WHO standard (18–20) and was supplied by Immuno.

Apolipoprotein B

The combination of antibodies was best as shown above. The standard had been calibrated against the proposed WHO standard and covered the range 0.125–4 mg/l (effective range up to 4 g/l). Samples were diluted 1 : 250, 1 : 500 and 1 : 1000 before being assayed. The same standard was used as for apolipoprotein A-I.

Apolipoprotein C-II : Apolipoprotein B

Samples were assayed neat using a serum calibrator for apolipoprotein C-II supplied by Immuno. The standard curve covered the range 3.5−105 mg/l.

Lipoprotein(a)

The standard used was for apolipoprotein(a) below, the specificity of the assay being determined by the use of anti-lipoprotein(a) on the solid phase and anti-apolipoprotein B as sandwich partner. Samples were diluted 1 : 10 and 1 : 50 before assay.

Apolipoprotein(a)

Samples were assayed using a 1 : 10 and 1 : 50 dilution using a standard curve which covered the range 0−80 mg/l (effective range 0−4000 mg/l). The standard material was from Immuno. The antibody combination shown above was the only one in which samples diluted linearly. The hope that free apolipoprotein(a) could be estimated from the difference between apolipoprotein(a) and lipoprotein(a) was not realisable due to the unknown amount of free apolipoprotein(a) in the standard used (information from Immuno).

Statistics

Non-parametric statistics were used throughout and correlations were performed with the *Spearman* rank correlation method. The median was used as the marker of central tendency and relevant percentiles for the range, as the distribution of apolipoprotein(a) and lipoprotein(a) concentrations was highly skewed (mean : median > 2.5).

Results and Discussion

Tables 2a−2b show quality assessment data for all five assays. The results of geometric dilution of selected samples are given in tables 3a and 3b.

The results show the large measuring range of the immunoluminometric assays, which allows very low concentrations of apolipoprotein C-II : apolipoprotein B complexes to be measured. Those patients presenting with metabolic disturbances, either with very low levels of this analyte (5) or with deficient lipoprotein lipase can be diagnosed reliably.

The precision of the assays is acceptable, although the accuracy, as far as this can be measured, must be questioned for the turbidimetric assay, especially in lipaemic sera. The relatively high coefficients of variation for the immunoluminometric assays for apolipoprotein A-I and apolipoprotein B are due to the large dilution factors coupled with the small sample volume.

Figure 1a shows the correlation between the turbidimetric method and the immunoluminometric assay in 92 sera from dialysis patients for apolipoprotein A-I. Figure 1b shows the same for apolipoprotein B. Correlations between the nephelometric assay for lipoprotein(a) and the immunoluminometric assays for apolipoprotein(a) and lipoprotein(a) are shown in figures 1c and 1d, respectively. Figure 1e shows the correlation and between both immunoluminometric assays.

No attempt was made to determine a reference range for healthy adults for any of the apolipoproteins, since there is an ongoing debate as to whether values for single apolipoproteins have any significance. From the correlation studies it can be seen that the assays presented here do not give very different results from the established routine serum nephelometric assay for lipoprotein(a). The results for apolipoproteins A-I and B were not as good.

The assays for apolipoproteins A-I, B, and C-II:B show a linear dilution pattern, showing that standards and samples behave in a similar way. Serum matrix effects were not apparent, but this may be due to the low concentrations of serum proteins, especially in the assays for apolipoprotein A-I and apolipoprotein B. The results of geometric dilution of selected samples for apolipoprotein(a) and lipoprotein(a) are given in table 3b. Stability studies for lipoprotein(a) and apolipoprotein(a) are shown in figure 2. Figure 3 shows the comparison between serum and EDTA-plasma lipoprotein(a) in 20 patients, with concentrations throughout the expected measuring range. Table 3b shows that not all sera dilute out as expected, which may reflect the different immunoreactivity of the small and large lipoprotein(a) molecules.

The results show that the immunoluminometric assays have a wide measuring range, which allows very low concentrations of lipoprotein(a) and apolipoprotein(a) to be measured.

The precision of the assays is acceptable, although the accuracy, as far as this can be measured, must be questioned for the nephelometric assay, especially in lipaemic sera. The nephelometric method often gave spurious results ranging from "under the detection limit of the assay" (due to the high extinction of the sample before reaction), to non-linear dilution curves; both effects were observed in lipaemic sera, and were not present in the immunoluminometric assays. The nephelometric assay was not able to measure lipoprotein(a) in plasma, all values being above 1000 mg/l. These findings point to the necessity of analysing serum when using nephelometric methods. Figure 3 shows that the immunoluminometric methods can be applied to serum or plasma.

The stability of lipoprotein(a) allows samples to be stored at 4 °C, samples stored in this way usually being stable for several weeks (fig. 2). Commercial control sera could be stored at 4 °C without loss of immunoreactive apolipoprotein(a) or lipoprotein(a). These observations are in accordance with those of *Gillery* et al.

Tab. 2a Quality assessment data for apolipoproteins A-I, B and C-II:B.

Apolipoprotein A-I

Standard curve data	Concentration (g/l)	Mean count integral	
	0	961	
	0.17	8349	
	0.34	25963	
	0.85	56974	
	1.70	102090	
Intra-assay precision (g/l)	No. of data pairs	Median CV (%)	
0.5–1.0	100	4.47	
1.0–1.5	144	4.03	
1.5–2.0	55	2.91	
2.0–2.5	15	2.08	
Inter-assay precision	No. of assays	Mean concentration (g/l)	CV (%)
Sample K1	22	0.55	9.42
Sample K2	21	1.16	9.87
Sample K3	22	1.79	8.83

Apolipoprotein B

Standard curve data	Concentration (g/l)	Mean count integral	
	0	897	
	0.143	2872	
	0.571	5608	
	1.14	20096	
	2.28	46842	
	4.57	77541	
Intra-assay precision (g/l)	No. of data pairs	Median CV (%)	
0.4–0.8	39	5.89	
0.8–1.2	93	3.26	
1.2–1.6	102	2.59	
1.6–2.4	27	2.89	
Inter-assay precision	No. of assays	Mean concentration (g/l)	CV (%)
Sample K1	19	0.49	12.2
Sample K2	19	0.85	9.21
Sample K3	18	1.47	8.74

Apolipoprotein C-II:B

Standard curve data	Concentration (mg/l)	Mean count integral	
	0	731	
	1.6	3176	
	6.5	11011	
	26	21869	
	52	27220	
	104	33495	

Tab. 2a *Apolipoprotein C-II:B* (Continued).

Intra-assay precision (mg/l)	No. of data pairs	Median CV (%)	
5– 30	114	2.79	
30– 60	46	2.80	
60–150	22	2.25	
Inter-assay precision	No. of assays	Mean concentration (g/l)	CV (%)
Sample K1	16	15.2	8.68
Sample K2	17	33.4	6.88
Sample K3	15	72.1	7.34

Tab. 2b Standard curve and quality control data for the apolipoprotein(a) and lipoprotein(a) assay.

Apolipoprotein(a)

Standard curve data	Concentration (mg/l)	Counts integral	
	0	1099	
	25	4129	
	50	9231	
	100	17171	
	200	40604	
	400	88305	
	800	133950	
Intra-assay precision	No. of data pairs	Median CV (%)	
Range (mg/l)			
5– 50	80	3.70	
50–250	96	3.01	
250–800	180	3.05	
Inter-assay precision	No. of assays	Mean concentration (mg/l)	CV (%)
Sample K11	24	298	6.23
Sample K12	24	149	7.12
Sample K13	24	38	7.34

Lipoprotein(a)

Standard curve data	Concentration (mg/l)	Counts integral	
	0	591	
	25	3408	
	50	7139	
	100	13699	
	200	38263	
	400	79690	
	800	124579	
Intra-assay precision	No. of data pairs	Median CV (%)	
Range (mg/l)			
5– 50	54	3.26	
50–250	71	3.15	
250–800	122	3.52	
Inter-assay precision	No. of assays	Mean concentration (mg/l)	CV (%)
Sample K11	15	351	6.75
Sample K12	15	162	6.43
Sample K13	15	34	6.97

Tab. 3a Effect of dilution of serum samples with assay buffer for apolipoproteins A-I, B and C-II:B.

Analyte – Serum dilution	Concentration measured	Concentration expected	Recovery (%)
Apolipoprotein A-I	(g/l)	(g/l)	
1 : 1000 (normal)	1.55	1.55	100
1 : 2000	0.764	0.775	95.4
1 : 4000	0.390	0.388	101
1 : 10000	0.181	0.155	117
Apolipoprotein B	(g/l)	(g/l)	
1 : 500 (normal)	0.771	0.771	100
1 : 1000	0.388	0.385	101
1 : 2500	0.162	0.154	105
1 : 5000	0.072	0.077	93.5
Apolipoprotein C-II	(mg/l)	(mg/l)	
Native	49.8	49.8	100
1 : 2	24.8	24.9	100
1 : 4	12.6	12.4	101
1 : 8	6.08	6.22	97.6

(21), who used a kinetic nephelometric assay, and *März* et al. (22) who used immunometric assays with radioisotopic and enzyme labelling, both groups having found no loss of immunoreactivity in human serum pools stored at 4 °C over a three week period.

The prediluted standard curve for (apo)lipoprotein(a) (1 : 10 dilution) in assay buffer is stable at 4 °C for at least 10 days, so that standards can be prepared once a week. Prediluted sera (1 : 10) can also be stored at 4 °C for at least a week without noticeable changes in the immunoreactivity of (apo)lipoprotein(a). When these standards or samples were stored at higher dilutions than 1 : 500 there was a loss of immunoreactivity after 7–10 days at 4 °C. No advantage was gained by storing the diluted samples/standards at −20 or −30 °C.

The excellent correlation between the two immunoluminometric assays and the poor correlation between the nephelometric assay and each of the immunoluminometric assays for apolipoprotein(a) and lipoprotein(a) reflects the practical problems of nephelometric measurements. The use of robust immunometric assays with signal detection is to be recommended for the determination of apolipoprotein(a) and lipoprotein(a) in serum. The non-concordance of results for apolipoprotein(a) and lipoprotein(a) is probably not due to free apolipoprotein(a) i. e. the fraction not bound to apolipoprotein B-100.

The different serum concentrations found for apolipoprotein(a) and lipoprotein(a) cannot be explained entirely by the presence of free apolipoprotein(a), nor by a cross-reactivity or displacement of apolipoprotein B-100 by apolipoprotein B-48 in the binding to the anti-apolipoprotein B on the solid phase. In view of the fact

Tab. 3b Geometric dilutions of selected sera and plasma for (apo)lipoprotein(a).

Analyte Dilution	Concentration measured (mg/l) Serum/Plasma	Concentration expected (mg/l) Serum/Plasma	Recovery (%) Serum/Plasma
Apolipoprotein(a)	Patient 1		
1 : 10 (normal)	162 /167	162 /167	100/100
1 : 20	79.8 / 83.1	81 / 83.5	99/100
1 : 160	37.9 / 40.7	40.5/ 41.8	91/ 97
1 : 80	17.6 / 21.4	20.2/ 20.9	87/102
1 : 160	9.72/ 9.57	10.1/ 10.4	96/ 92
Lipoprotein(a)	Patient 2*)		
1 : 10 (normal)	1046	1046	100
1 : 20	515	523	98
1 : 40	264	262	100
1 : 80	136	131	105
1 : 160	71	65.5	109
Lipoprotein(a)	Patient 3*)		
1 : 10	1420	1420	100
1 : 20	795	710	112
1 : 40	420	355	118
1 : 80	250	178	145
1 : 160	155	89	174

*) Serum only

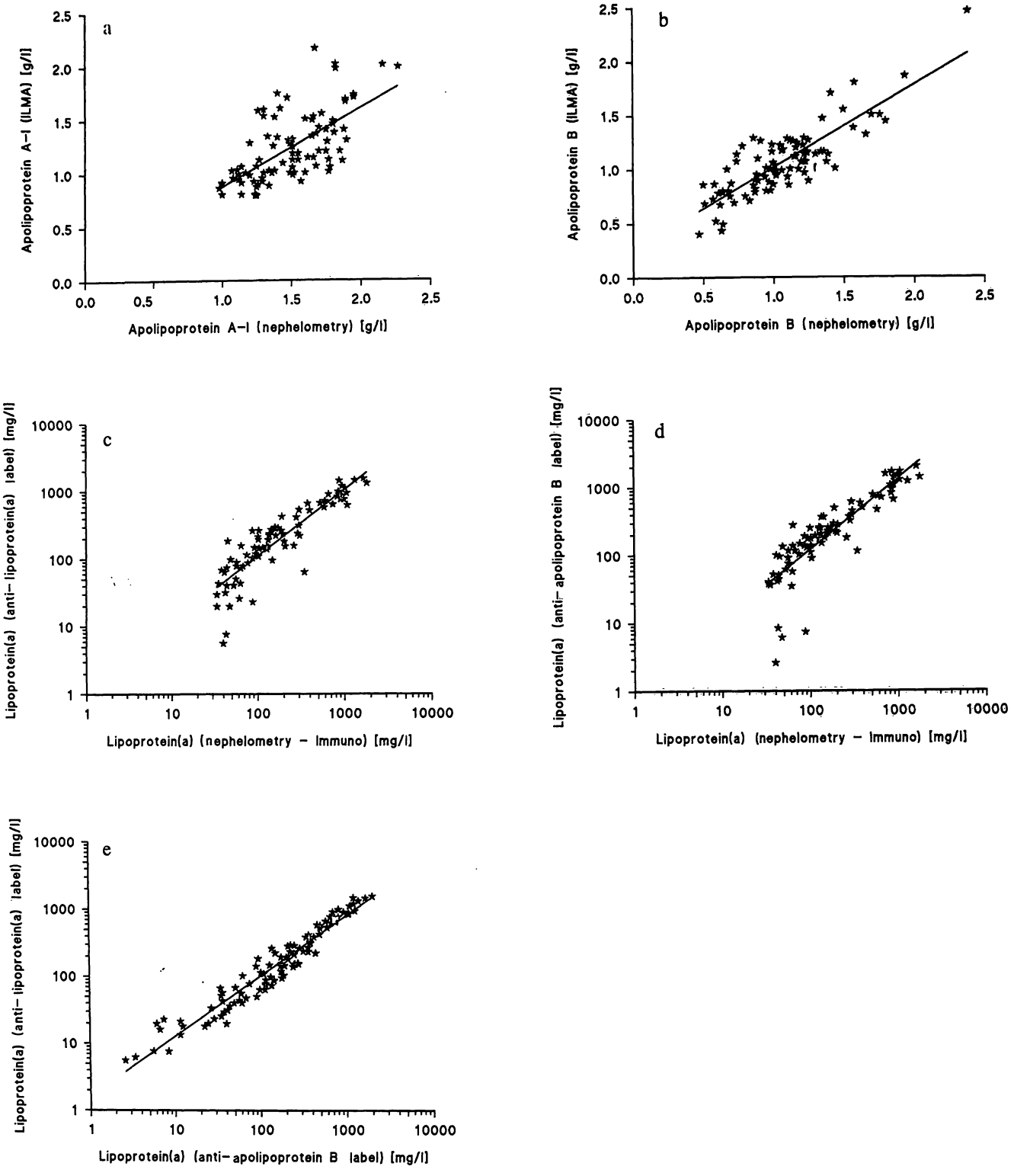

Fig. 1 Method comparison results.
a) Comparison of the turbidimetric assay (x) with the immunoluminometric assay (y) for apolipoprotein A-I in 92 samples from dialysis patients.
The correlation coefficient r = 0.65.
The regression line equation is: y = 0.73x + 0.14.
b) Comparison of the turbidimetric assay (x) with the immunoluminometric assay (y) for apolipoprotein B.
The correlation coefficient r = 0.84.
The regression line equation is: y = 0.76x + 0.26.
c) Comparison of the nephelometric method for Lp(a) (x) with the immunoluminometric assay for lipoprotein(a) (y, anti-lipoprotein(a) label) on 83 samples.
The correlation coefficient r = 0.888.
The regression line equation is: log (y) = 0.981 log (x) + 0.073.
(21 samples, which lay below the detection limit of the nephelometric method, were excluded from the comparison.)
d) Comparison of the nephelometric method for Lp(a) (x) with the immunoluminometric assay for lipoprotein(a) (y, anti-apolipoprotein B label) on 83 samples. The correlation coefficient r = 0.868.
The regression line equation is: log (y) = 1.04 log (x) + 0.005.
(21 samples, which lay below the detection limit of the nephelometric method, were excluded from the comparison.)
e) Comparison of the immunoluminometric assays for lipoprotein(a) using anti-apolipoprotein B label (x) and anti-lipoprotein(a) label (y) on 104 samples.
The correlation coefficient r = 0.965.
The regression of the equation is: log (y) = 0.894 log (x) + 0.210.

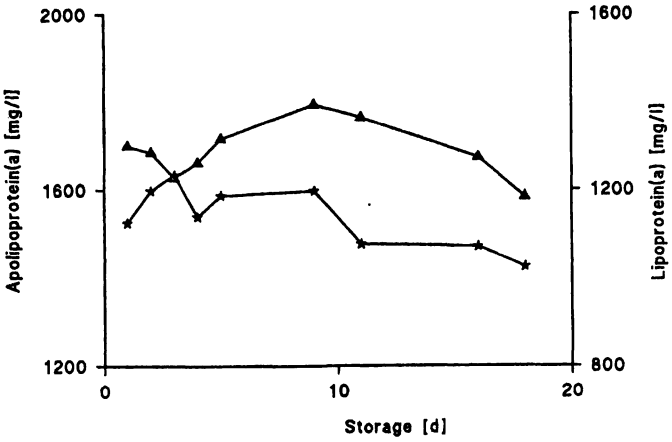

Fig. 2 Stability of a serum sample stored at 4 °C over a three week period. Comparison between results from the nephelometric method for Lp(a) (★) and the immunoluminometric method for apolipoprotein(a) (▲).
The concentrations of other lipid analytes in this sample were:
cholesterol – 7.8 mmol/l,
triacylglycerols – 0.92 mmol/l,
HDL-cholesterol – 2.5 mmol/l,
LDL-cholesterol – 4.9 mmol/l,
lipase – 1.51 μmol · l^{-1} s^{-1}.

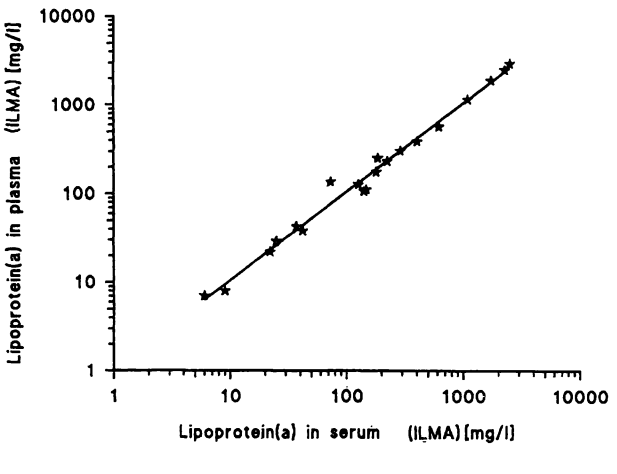

Fig. 3 Comparison of lipoprotein(a) in serum and plasma from 20 patients using the immunoluminometric assay.
The correlation coefficient r = 0.993.
The slope of the regression line is: log (y) = 0.998 log (x) + 0.022.

that apolipoprotein B-100 is found in lipoprotein(a), but not in apolipoprotein(a), and free apolipoprotein(a) may be present in serum in different amounts, the results should, if anything, be higher for apolipoprotein(a) than for lipoprotein(a). This was not observed (see fig. 1e). The results from the present study agree however with those of *März* and coworkers, who found a much greater discrepancy between plasma lipoprotein(a) and apolipoprotein(a) using commercial kits, the slope of the regression line being between 0.34 and 0.54, depending upon the calculation method (14). As the anti apolipoprotein(a) antisera are both polyclonal, it is not to be expected that they are directed against a single epitope, even when the manufacturers state that they are specific for apolipoprotein(a), antibodies against plasminogen having been removed by immunoadsorption. The antibodies are specific for conformational epitopes, which most probably differ according to the molecular structure of apolipoprotein(a). The differences in the dilution patterns of different sera support this theory.

The median concentration of lipoprotein(a) from 147 non-selected hospitalised patients was 136 mg/l for the nephelometric method and 179 mg/l for the immunoluminometric assay. The median concentration of apolipoprotein(a) was 151 mg/l for the same samples. These values are comparable with those reported by *Panteghini & Pagani* (23), who reported a mean of 140 mg/l for healthy individuals for an immunoluminometric assay for lipoprotein(a). The comparison of values from different methods for lipoprotein(a) is problematic as it depends upon the material used for standardisation.

The immunometric assays are better suited to the measurement of lipoprotein(a) and apolipoprotein(a), as they are designed to measure the molecule specifically and are not influenced by factors such as lipaemia and the presence of coagulation factors, both of which may influence nephelometric or turbidometric assays. The disadvantage of the immunometric assays is the need for dilution of the sample, although this may dilute out any adverse serum-matrix effects that may be present. The assays are suitable for partial automation and are capable of processing several hundred samples per day, a point worth noting when carrying out epidemiological studies or when screening large groups.

The quality assessment is acceptable in terms of inter- and intra-assay precision. The question of accuracy is still open, due to the absence of an accepted international reference preparation or standard. The microheterogeneity of lipoprotein(a) and the different genetic variants will make the preparation of a single standard difficult, especially if the antibodies used only recognise certain genetic variants or conformational epitopes of apolipoprotein(a) (24). The knowledge of antibody specificity is important in the determination of lipoprotein(a), so that the different forms of the lipoprotein(a) molecule can quantified separately. This would be helpful if the different forms of lipoprotein(a) were conclusively shown to have different atherogenic properties.

Further difficulties in assessing the value of studies on lipoprotein(a) lie in the incorrect use of statistics by many groups, where, despite the skewed distribution of values, parametric statistics have been used (25).

The correlation coefficients, although statistically significant, were not good, especially for apolipoprotein A-I, and many reflect the difference in assay design

(competitive versus sandwich methodology) as well as the standard used and the effect of the sample material itself. Samples from dialysis patients were chosen, as it is known that serum from such patients often presents analytical problems, especially in nephelometric and turbidimetric assays due to the native turbidity of the sample. The standard from Roche used on the Cobas MIRA gave a non-linear dilution curve for apolipoprotein A-I in the immunoluminometric assay, and values which were up to three times too low, when compared with those from the standard from Immuno (data not presented here).

The non-standardisation of methods for the apolipoproteins limits the comparability of results obtained by different methods, so that the value of many studies must be questioned. Even with the introduction of an international standard for apolipoprotein A-I and apolipoprotein B (18–20) it is not to be expected the inter-method comparison will be better. This has been shown repeatedly, especially for proteohormones and polypeptides. In the comparison of 29 methods for apolipoprotein A-I using the proposed new international standard (20) it was explicitly stated that the values assigned by companies to their secondary standards only holds for the system used. Any change in the system may give rise to other values (20). This shows the limits of standardisation by use of a common antigen. Twenty-eight of the 29 methods used in this study to determine apolipoprotein A-I were one-site competitive turbidimetric or nephelometric methods, the remaining assay using radial immunodiffusion. It should be noted, the latter assay gave results which deviated most and had the lowest correlation coefficient.

The results for apolipoprotein C-II could not be compared with another method as none was available. An indirect confirmation that the assay measured in the expected range was provided by reference ranges for apolipoprotein C-II (30–80 mg/l) from other sources (26, 27). In this study, the median concentration of 176 dialysis patient samples was 30 mg/l (range 4.1–176 mg/l). The median concentration of 100 non-lipaemic serum samples from healthy adults was 37 mg/l (range 9.6–85 mg/l). There was no sex-linked difference. This represented the amount of apolipoprotein C-II complexed with apolipoprotein B; it was not possible to set up an immunometric method for apolipoprotein C-II alone. The lower values may be due to the incomplete determination of apolipoprotein C-II, i. e. that not complexed with apolipoprotein B, or to the specificity of the sandwich method or the standard used. The method and results for apolipoprotein C-II:B complexes have been included to show that it is possible to measure apolipoprotein complexes. Although this assay is of minimum routine use, it may be of interest in those centres concerned with diagnosis of lipoprotein abnormalities, especially in renal dialysis patients, many of whom are known to have severe lipid disorders.

This short communication is intended to demonstrate the use of robust and reliable immunometric methodology in the field of lipid metabolism, especially where large numbers of samples can be analysed economically using commercially available reagents not in kit form. Such assays provide consistent results over a long period, provided sufficient antisera is purchased. This is especially important in long-term epidemiological studies, where methodological variation must be excluded for an optimal interpretation of the data obtained.

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